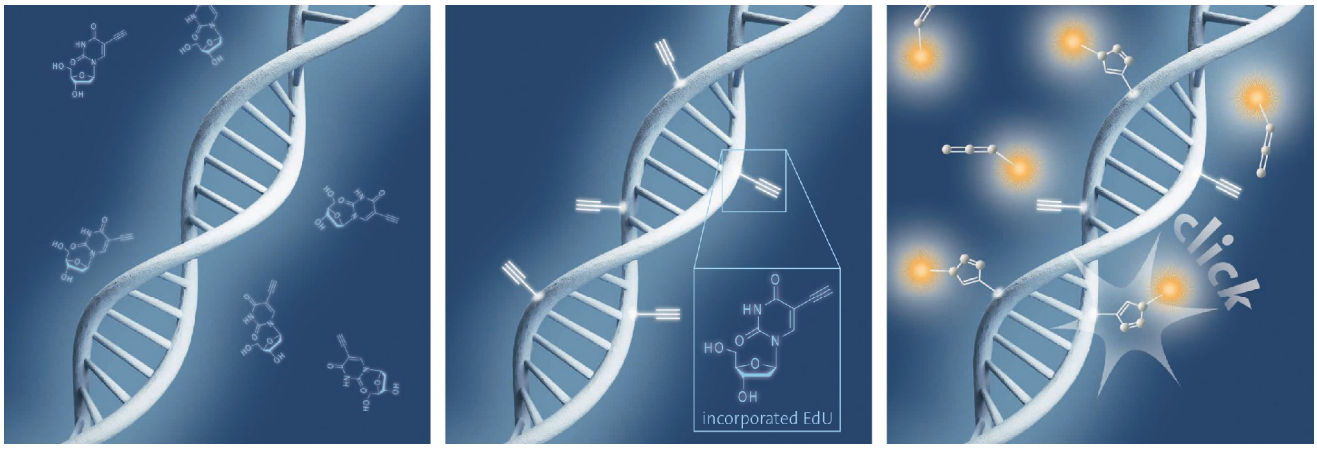
USER MANUAL

Oligo Click M

ROTI®kit for DNA labeling



Oligo Click M

ROTI®kit for DNA labeling

For Click Chemistry labeling of up to 100 nmol oligonucleotide containing 1 to 10 alkynes.

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Cautions:

Activator: Warning H226-H319-H335
P210-P280-P303+P361+P353-P305+P351+P338-P312a

Solvent ROTI®click grade: Warning H226-H319-H335
P210-P280-P303+P361+P353-P305+P351+P338-P312a

MSDS: the appropriate MSDS can be downloaded from our website www.carlroth.com.

Literature citation:
When describing a procedure for publication using this product, please refer to it as the *Carl Roth's ROTI®kit for DNA labeling (Oligo Click M)*.

We recommend using the following general protocol for click chemistry labeling of alkyne-modified oligonucleotides (from 10 to 100 nmol) with Label-Azides provided by Carl Roth GmbH + Co. KG.

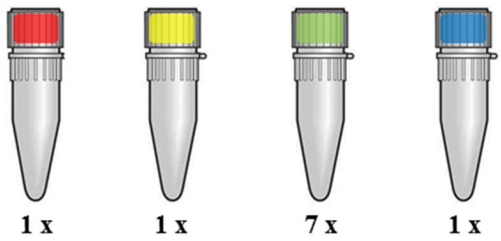
Protocol

A. General considerations

- This protocol is optimized for the labeling of up to **100 nmol** of a single or double alkyne-modified oligonucleotide via copper(I)-catalyzed azide-alkyne cycloaddition (CuAAc; Click Chemistry).
- The Reactor M vial contains a stable **heterogeneous catalyst**, which won't be dissolved during the reaction.
- The labeling reaction works more efficiently with concentrated solutions of alkynes (oligo) and azides (Label-Azide, L-N_3).
- The best way to carry out the click reaction is to mix the oligo and the Label-Azide in a minimal amount of solvent.
- The click reaction is normally accelerated by elevated temperatures and can be finished in 30 min when the reaction temperature is 45 °C. Low reaction temperatures (e.g. 4 °C) can be applied as well in combination with longer reaction time.
- The reaction time depends on: a) concentration of azide and oligo in the solution; b) reaction temperature; c) stirring and/or mixing of the solution; d) azide steric demand for double-labeling reactions. In the latter case use a prolonged (4 h) reaction time.

B. Materials and storage conditions for up to seven (7) independent labeling reactions provided with the Oligo Click M kit.

Vial colour	Quantity	Name	Amount	Storage
red	1	Azide	1 mg	Dark, -20 °C
yellow	1	Activator	20 µL	-20 °C
green	7	Reactor M	N.A.	RT
blue	1	Solvent	1 mL	RT

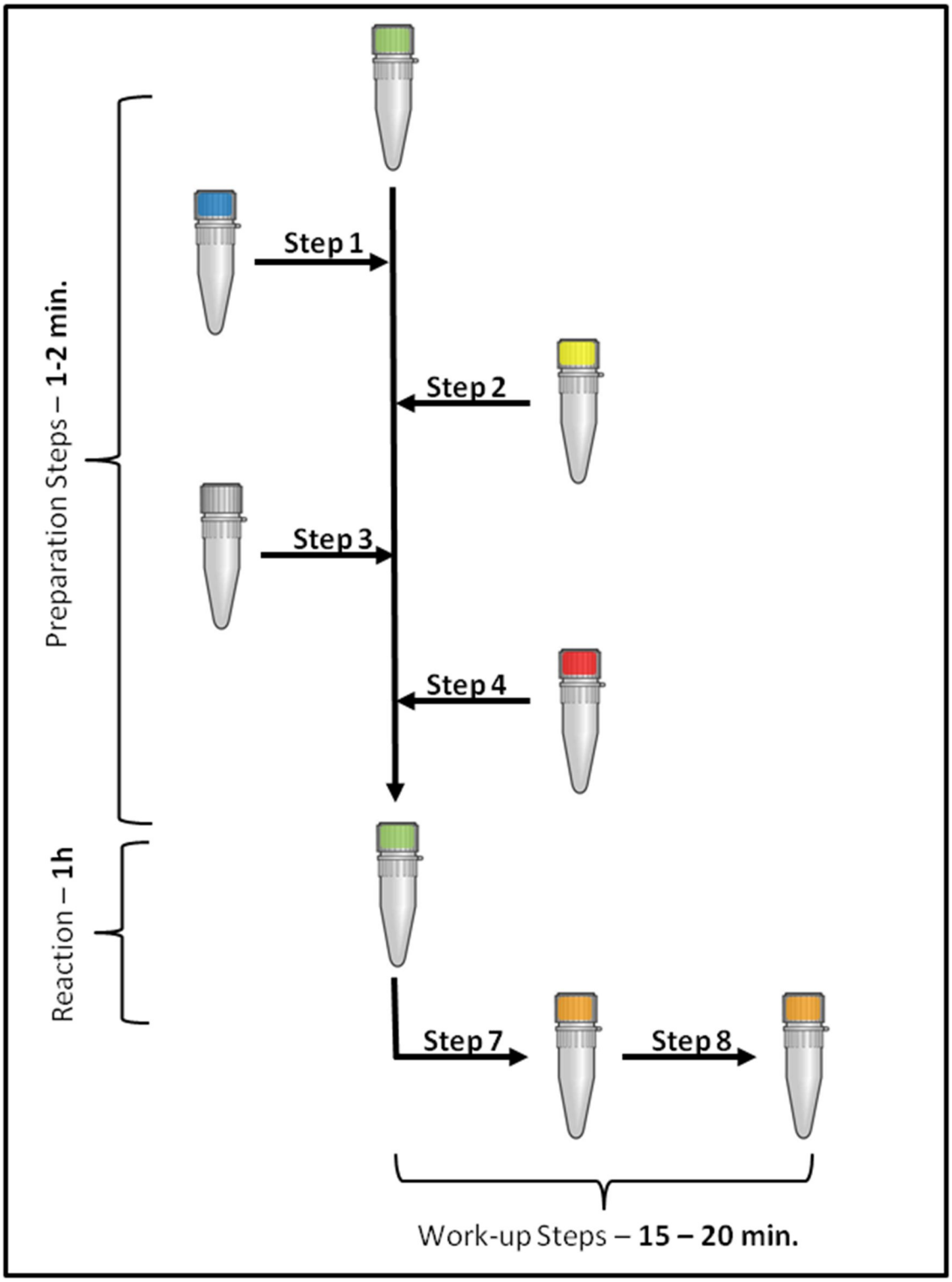


C. Required Material and Equipment – not provided with this kit

- Alkyne-modified oligonucleotide or Alkyne-modified PCR fragment
- Centrifuge (optional refrigerated)
- Microcentrifuge tubes

- Thermomixer (optional)
- Ethanol 95%
- 3 M Sodium-acetate solution (3 M NaOAc) or ammonium-acetate 3 M NH_4OAc.

D. Work Flow



Vial colour	Name
red	Azide
yellow	Activator
green	Reactor M
blue	Solvent

Step 1

- Take 6 µL from the blue vial
- Add to the green vial

Step 2

- Take 2 µL from the yellow vial
- Add to the green vial

Step 3

- Add the proper amount of oligonucleotide or DNA into the green vial

Step 4

- Take the proper amount from the red vial
- Add to the green vial

Step 5

- Gently mix the green vial
- Shortly centrifuge the green vial

Step 6

- Heat to 45 °C under shaking for 1 h
- Alternatively, place the green vial in a thermo bath for 1 h at 45 °C

Step 7

- Transfer the liquid phase in a new empty vial
- Wash the green vial with 60 µL NaOAc 3 M
- Transfer the liquid phases from the green vial into the new empty vial

Step 8

- Add chilled EtOH 95%
- Proceed with your preferred work-up

E. Click protocol for Oligonucleotide and PCR labeling

1. Preparation of the Oligonucleotide or PCR fragment solution (not provided with the kit)

Dissolve the oligonucleotide in the appropriate amount of water to adjust to a 0.1 - 1 mM solution and centrifuge shortly. (Also different concentrations can be used, see Reaction Table at page 7).

or

Dissolve the PCR fragment in an appropriate amount of water or buffer (**avoid** EDTA and EDTA-containing buffers) to adjust to ca. 50 – 150 ng/µL solution.

2. Preparation of a 10 mM Label-Azide (L-N_3) solution[1]

(Select your preferred Oligo Click / Azide combination from www.carlroth.com)

2.1 Take the **red vial** with 1 mg of your selected azide L-N_3 out of the freezer and slowly warm up to room temperature.

2.2 Centrifuge shortly to place all L-N_3 on the bottom of the **red vial**.

2.3 Pipette (100,000 / $MW_{L\text{-}N3}$) µL of the solvent (**blue vial**)[2] into the **red vial** containing the Label-Azide.[3]

2.4 Vortex the **red vial** until the Label-Azide is dissolved completely.

2.5 Centrifuge shortly.

3. Performing the click reaction (1-2 min. preparation + 1 h reaction)

(Be aware that the catalyst is solid and will not be dissolved during the click reaction!)

[Step 1] Pipette **6 µL** of the solvent (**blue vial**) into the **green vial** with the catalyst

[Step 2] Pipette **2 µL** of the activator (**yellow vial**) into the **green vial** from Step 1

[Step 3] Pipette the appropriate amount of the oligo or DNA solution[4] into the **green vial** from Step 2

[Step 4] Pipette the correct amount[5] of Label-Azide solution (L-N_3 **red vial**) reported in the Reaction Table at page 7 into the **green vial** from Step 3

[Step 5] Vortex the **green vial** from Step 4 for 10 sec. Centrifuge shortly

[Step 6] Place the **green vial** from Step 5 in a thermomixer at 45 °C for 1 h under gentle shaking (do not exceed 700 rpi) or in a water bath at 45 °C for 1 h. You can run the

[1] This preparation is valid for Label-Azides (not included in this kit) soluble in DMSO. You can also use pure water or other solvents compatible with the Label-Azide you selected (see azides under www.carlroth.com)
[2] This solvent contains a DMSO/*t*-BuOH mixture. Download the MSDS from www.carlroth.com (Art.No. 7815).
[3] The molecular weight $MW_{L\text{-}N3}$ is given on page 13. See also the calculation sheet on page 8.
[4] See "Minimal Oligo Conc." and "Maximal Reaction Volume" in Reaction Table on page 7.
[5] See Reaction Table at page 7 or the calculation sheet on pages 8-10.

reaction at room temperature (RT) as well. In this case use a prolonged reaction time (2-4 h).

IMPORTANT: Provide always some mixing over the reaction time. The catalyst in the green vial will not be dissolved!

4. Work up (15 – 20 min.)

[Step 7]

4.1 Transfer only the liquid phase into a **new empty vial**

4.2 Wash the **green vial** containing the solid catalyst with 60 µL of 3M NaOAc

4.3 Collect only the liquid phase from point 4.2 in the **new empty vial** containing your labeled-oligonucleotide from step 4.1

Proceed with your preferred DNA precipitation or continue with point 5:

5. Precipitation protocol

[Step 8]

5.1 Add 1 mL cold ethanol 95%

5.2 Centrifuge for at least 15 min at 4 °C or cool for 1 h at -20 °C and then centrifuge

5.3 Remove the supernatant and dry the residue on air

5.4 Re-dissolve the pellets in the desired amount of water or buffer

Your labeled-oligonucleotide or DNA is ready for your experiment / assay. The final product may contain traces of free Label-Azide, although most of the reagents have been removed during the precipitation step. Applicable purification methods: 1. Desalting. 2. RP-HPLC. 3. Gel Electrophoresis.

Reaction Table:

Use the following table to calculate the amount of reagents (Activator, Solvent and Azide) you need in your oligonucleotide labeling click reactions you in a fast and very reliable way.[6]

You will need different amounts of Label-Azide – "*µL Azide (Red)*" column - depending on the amount of oligonucleotide – "*Oligo nmol range*" column - and the amount of alkynes present in your sequence – "*Alkyne content range*" column.

Add the reagents as described in Point 3 of this protocol.

Oligo nmol range	Alkyne content range	µL Activator (Yellow)	µl Solvent (Blue)	µL Azide (Red)	Reactor (Green)	Minimal Oligo Conc.	Maximal reaction volume in µL
11 - 30	*For a 22mer this range corresponds to **2.5 – 6.6 OD** or **73 – 200 µg***						
	1 - 2	**2**	**6**	**12**	M	0.1 mM	150
	3 - 6	**2**	**6**	**36**	M	0.1 mM	300
	7 - 10	**2**	**6**	**60**	M	0.1 mM	300
31 - 50	*For a 22mer this range corresponds to **7.0 - 11 OD** or **205 - 330 µg***						
	1 - 2	**2**	**6**	**20**	M	0.1 mM	300
	3 - 6	**2**	**6**	**60**	M	0.1 mM	300
	7 - 10	**2**	**6**	**100**	M	0.1 mM	300
51 - 70	*For a 22mer this range corresponds to **11 - 16 OD** or **337 - 462 µg***						
	1 - 2	**2**	**6**	**28**	M	0.1 mM	300
	3 - 6	**2**	**6**	**84**	M	0.1 mM	300
	7 - 10	**2**	**6**	**140**	M	1.0 mM	300
71-100	*For a 22mer this range corresponds to **16 - 22 OD** or **337 - 470 µg***						
	1 - 2	**2**	**6**	**40**	M	0.1 mM	300
	3 - 6	**2**	**6**	**120**	M	0.1 mM	300
	7 - 10	**2**	**6**	**200**	M	1.0 mM	300

[6] For a detailed calculation see page 8 of this user manual. Use the Azide Table on page 10 in order to minimize the amount of Label-Azide required in your labeling reaction.

Appendix

F. Calculation Sheet

1 Preparation of a 10 mM Label-Azide (L-N_3) Solution

To calculate the amount of solvent V_L in µL to be added to 1 mg of Label-Azide (L-N_3) to prepare a 10mM solution divide 100,000 by the molecular weight of the Label-Azide ($MW_{L\text{-}N3}$).

E.g.:

- m = Label-Azide = FAM-N_3 1 mg
- $MW_{L\text{-}N3}$ = 458.4 g/mol
- V_L = 100,000 / 458.4 = 218.2 µL
- c_{azide} = 10 mM

1.1 Take the **red vial** with 1 mg Label-Azide out of the freezer and slowly warm up to room temperature.

1.2 Centrifuge shortly to place all the Label-Azide on the bottom of the vial.

1.3 Pipette V_L (µL calculated in 1) of solvent from the **blue vial** into the **red vial** with the Label-Azide.

1.4 Vortex the **red vial** until the Label-Azide is dissolved completely.

1.5 Centrifuge shortly. This solution can be stored at -20 °C in the dark for several months (refer to the Label-Azide Data-Sheet). The azide functionality is very stable and does not hydrolyze in water.

G. Click reaction calculation sheet

Use the **Reaction Table** on page 7 to read out the amount of Label-Azide (L-N_3) to be used in your experiment. Use the **Azide Table** on page 10 if you need to minimize the amount of Label-Azide used in your labeling reaction. Below you can read how you can calculate those values yourself:

1. *For oligonucleotide labeling*:

1.1 Calculate the amount of oligonucleotide n_{oligo} in nmol

- n_{oligo} [nmol] = m [ng] / MW [g/mol]
- n [nmol] = c [mM] x V [µL]

1.2 If you have a concentration c [ng/µL] divide this value by the molecular weight MW [g/mol] of your oligo in order to obtain the total concentration in nmol/µl. Multiply this value by the total volume in µl to obtain the total amount of your oligo n_{oligo} in nmol.

Example:
oligonucleotide containing three (3) alkynes and the following specifications:

- c_{oligo} = 250 ng/µL
- MW_{oligo} = 6500 g/mol

- Total volume = V_{oligo} = 150 µL
- Total amount = n_{oligo} = (250 / 6500) x 150 = 5.8 nmol

1.3 Multiply n_{oligo} by the total amount of incorporated alkynes in order to obtain $n_{alkynes}$ in nmol.

- Oligo containing 3 alkynes
- n_{oligo} = 5.8 nmol
- $n_{alkynes}$ = 5.8 x 3 = 17.4 nmol

1.4 The click reaction requires only two equivalents of azide. Multiply $n_{alkynes}$ x 2 to obtain n_{azide} in nmol.

- n_{azide} = 17.4 x 2 = 34.8 nmol

1.5 Divide n_{azide} by the azide concentration c_{azide} = 10 mM in order to obtain the amount of azide (V_{azide} in µL) to be used in the reaction.

- $V_{azide} = n_{azide} / c_{azide}$ = 34.8 / 10 = 3.5 µL
- Use 3.5 µL of Label-Azide 10 mM in your click reaction.

2. *For PCR labeling:*

Calculate the amount of Azide (L-N_3) that you want to use for labeling your alkyne-modified DNA. The final labeling rate of the DNA can be tuned by the amount of azide used and has to be adjusted for every new DNA template.

2.1 Measure the DNA concentration c_{DNA} [ng/µL] after PCR workup with a photometer.

2.2 Calculate the molecular weight MW (g/mol) of your DNA template (MW_{DNA}):

MW_{DNA} [g/mol] = 600 g/mol x bp

- 600 g/mol is the average mass of a basepair
- bp = number of basepairs in your DNA template

2.3 Calculate the total amount of DNA n_{DNA} in nmol present in your sample:

n_{DNA} [nmol] = c_{DNA} [ng/µL] x V_{DNA} [µL] / MW [g/mol]

- c_{DNA} [ng/µL]: measured in 2.1
- MW_{DNA} [g/mol]: calculated in 2.2
- V_{DNA} [µL] = volume of your sample (measure it with a pipette)

2.4 Calculate the total amount of terminal alkyne modifications $n_{alkynes}$ in nmol in your DNA. This amount corresponds to the amount of Thymidines in your DNA if dTTP was replaced by **C8-Alkyne-dUTP** during PCR:

$n_{alkynes}$ [nmol] = [(bp x AT-content %) / 100] x n_{DNA} [nmol]

- bp = number of basepairs in your DNA template
- AT-content % = percentage of A's and T's in your DNA
- n_{DNA} (nmol) = calculated in 2.3

If dCTP was replaced by **C8-Alkyne-dCTP** during PCR then calculate $n_{alkynes}$ in nmol in your DNA as follow:

$n_{alkynes}$ [nmol] = (bp x GC-content %) / 100 x n_{DNA} [nmol]

- bp = number of basepairs in your DNA template
- GC-content % = percentage of G's and C's in your DNA
- n_{DNA} [nmol] = calculated in 2.3

2.5 Calculate the amount of Label-Azide n_{azide} in nmol for labeling the alkyne-modified DNA. Labeling rates depend on the amount of Label-Azide applied. Normally 1 – 30 equivalents of azide are used, resulting in labeling rates of up to 20 % and more!

n_{azide} [nmol] = $n_{alkynes}$ [nmol] x k

- $n_{alkynes}$ [nmol] = calculated in 2.4
- k = equivalents of azide (normally 1 – 30)

V_{azide} (Label-Azide; 10 mM) = n_{azide} [nmol] / 10 nmol/µL

Add V_{azide} [µL] to your click reaction.

H. Azide Table

Use these tables to read out the **minimum amount** of Label-Azide needed in your labeling click reaction, in order to reduce the Label-Azide consumption when needed.

For example, if you have 65 nmol of an oligonucleotide (nmol Oligo = 65) containing 4 alkynes in the sequence (Nr. of Alkynes = 4) then use 52 µL of the Label-Azide 10 mM solution.[7]

Nr. of Alkynes	1	2	3	4	5	6	7	8	9	10
nmol Oligo	µL Azide	µL Azide	µL Azide	µL Azide	µL Azide	µL Azide	µL Azide	µL Azide	µL Azide	µL Azide
15	3	6	9	12	15	18	21	24	27	30
20	4	8	12	16	20	24	28	32	36	40
25	5	10	15	20	25	30	35	40	45	50
30	6	12	18	24	30	36	42	48	54	60
35	7	14	21	28	35	42	49	56	63	70
40	8	16	24	32	40	48	56	64	72	80
45	9	18	27	36	45	54	63	72	81	90
50	10	20	30	40	50	60	70	80	90	100
55	11	22	33	44	55	66	77	88	99	110
60	12	24	36	48	60	72	84	96	108	120
65	13	26	39	52	65	78	91	104	117	130
70	14	28	42	56	70	84	98	112	126	140
75	15	30	45	60	75	90	105	120	135	150
80	16	32	48	64	80	96	112	128	144	160
85	17	34	51	68	85	102	119	136	153	170
90	18	36	54	72	90	108	126	144	162	180
95	19	38	57	76	95	114	133	152	171	190
100	20	40	60	80	100	120	140	160	180	200

[7] The amount of Label-Azide reported in the Reaction Table at page 7 are for this example 84 µL, which cover the range between 51 and 70 nmol oligo containing from 3 to 6 alkynes in the sequence.

Troubleshooting

If the labeling is not complete then increase the reaction time and eventually the reaction temperature (recommended for multi labeling reactions and/or for azides with high steric demand).

- ***Your notes*:**

Ordering information:

(for detailed kit content see Table under B.)

ROTI®kits for DNA labeling:

Product number	Product	Used Label-Azide	Reactor
7764.1	Oligo Click S Reload	-	9 x 2,5 mg
7765.1	Oligo Click M Reload	-	9 x 5 mg
7766.1	Oligo Click S-488	6-FAM-Azide $MW_{L\text{-}N3}$ = 458.43 g/mol	7 x 2,5 mg
7767.1	Oligo Click M-488	6-FAM-Azide $MW_{L\text{-}N3}$ = 458.43 g/mol	7 x 5 mg
7769.1	Oligo Click S-555	5-TAMRA-Azide $MW_{L\text{-}N3}$ = 512.56 g/mol	7 x 2,5 mg
7770.1	Oligo Click M-555	5-TAMRA-Azide $MW_{L\text{-}N3}$ = 512.56 g/mol	7 x 5 mg
7771.1	Oligo Click S-Biotin	Biotin Azide $MW_{L\text{-}N3}$ = 326.42 g/mol	7 x 2,5 mg
7772.1	Oligo Click M-Biotin	Biotin Azide $MW_{L\text{-}N3}$ = 326.42 g/mol	7 x 5 mg

To place your order, please contact us:

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